

In the matter of US Patent Application No. 10/535,433

I, Alessandro Vitale, currently residing at via Ciro Menotti 33, 20133 Milano, Italy, European Union, do hereby declare:

1. I am not one of the inventors or applicant of US 10/535,433, a copy of which I have read and understood. I have no commercial relationship with the inventors or applicant of US 10/535,433. Between April 1996 and August 1998 Dr Lorenzo Frigerio, an inventor of US 10/535,433, worked as a post-doctoral research associate in my laboratory at the Institute for Plant Biology and Biotechnology, National Research Council, Milano, Italy.

2. I am an expert in the field of the invention. My curriculum vitae is enclosed.

3. I have published over 80 scientific papers and book chapters. These are listed in the attached Annex.

4. In the Official Action from the United States Patent and Trademark Office, dated 21 June 2007, the Examiner believes that the claims contain subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. I disagree. The Examiner also believes that the application does not provide sufficient information to allow one skilled in the art to repeat the invention across the full breadth of the claims. I disagree.

I declare that one skilled in the art as of the filing date of the present application (May 18, 2005) would have been able to repeat the claimed invention across its full breadth using the teaching of the application as filed in combination with his common general knowledge. The inventor has identified the underlying principle to whether an immunoglobulin expressed in a plant is to be secreted or directed towards a vacuole. One skilled in the art would be able to generate mutations in the C terminal 18 amino acids of an immunoglobulin and then test whether the mutated immunoglobulin possessed a vacuolar targeting signal (*i.e.* the 'principle biological property' of the claimed invention). There is not an infinite number of possible mutations. The claimed mutations lie within the C-terminal 18 amino acids of the immunoglobulin. There is no need to provide one skilled in the art with each and every possible nucleotide sequence and amino acid sequence. I believe that US 10/535,433 provides a sufficient number of representative species of sequences to demonstrate that the invention works across the whole claimed genus of sequences.

Furthermore, the skilled person could easily test whether the mutated immunoglobulin retained the antigen-binding specificity of the parent (*i.e.* non-mutated) immunoglobulin. Such tests were within the skills of undergraduate biology students as of May 18, 2005, hence the skilled person would have no difficulty in carrying out such a test.

I also believe the application as filed shows that the inventor, as of the filing date of the application, had possession of the full scope of the invention as now claimed. For example, page 8, line 1 through the top of page 10 of the application describes the broad scope of the invention and various aspects of modifications to the C-terminus 18 amino acids of the heavy chain. While the text does not specifically delineate each and every possible individual modification, I believe the application as filed shows that the inventor had possession of the claimed method, including modifying the nucleotide sequence that encodes the

immunoglobulin heavy chain to form a modified nucleotide sequence, wherein the modifying is in the region of the nucleotide sequence encoding the C-terminus 18 amino acids of the immunoglobulin heavy chain to remove, or reduce the effectiveness of, one or more vacuolar targeting signal sequences of the encoded immunoglobulin heavy chain.

In summary, I declare that the claims clearly describe the invention and that one skilled in the art would be able to repeat the claimed invention without undue experimentation.

5. The Examiner believes that the claimed invention is obvious in view of the teachings of Frigerio *et al* (Plant Physiology 123: 1483-1493 (August 2000) (hereinafter "Frigerio")) in combination with one or more of Vitale and Raikhel (Trends in Plant Science 4(4): 149-155 (April 1999)) (hereinafter "Vitale"), Koide *et al* (Plant Cell Physiol. 40(11): 1152-1159 (1999)) (hereinafter "Koide"), Matsuoka and Neuhaus (J. Exp. Bot. 50: 165-174 (1999)) (hereinafter ("Matsuoka")). I disagree.

I declare that I am a co-author of the Frigerio and Vitale publication. As a person skilled in the art of the field of this invention at the time the present application was filed, I declare that, prior to the present application, it was not known or obvious that proteins originating from mammals, in particular humans, that are synthesized by the secretory pathway, contain cryptic sorting signals that cause those mammalian proteins, in particular human proteins, to be targeted to a particular location within the cell when expressed in a plant. The only known exception is default secretion, which does not require additional signals apart from the signal peptide to enter the secretory pathway.

I declare that the constructs and methods of the present invention are novel and not obvious as compared to the methods and antibodies disclosed in Frigerio *et al* (Plant Physiology 123: 1483-1493 (August 2000) (hereinafter "Frigerio")), Vitale and Raikhel (Trends in Plant Science 4(4): 149-155 (April 1999)) (hereinafter "Vitale"), Koide *et al* (Plant Cell Physiol. 40(11): 1152-1159 (1999)) (hereinafter "Koide"), Matsuoka and Neuhaus (J. Exp. Bot. 50: 165-174 (1999)) (hereinafter ("Matsuoka")).

I declare that Frigerio relates to the expression of immunoglobulin, *i.e.* a mammalian protein, in a plant. Frigerio identifies that the protein is delivered to two different sites in the plant: a proportion of molecules is transported to the vacuole and a proportion of molecules is secreted. However, Frigerio does not identify the underlying reason for the protein being delivered to two different sites of the plant. Frigerio does not identify a cryptic signal in the amino acid sequence of an immunoglobulin. Instead, Frigerio predicts that the reason might be:

- (i) Due to autophagy by the vacuole, through unknown recognition events
- (ii) Due to the presence of thiol-mediated retention in the plant ER [endoplasmic reticulum], similar to thiol-mediated retention reported in mammalian cells (see page 1489, left hand column).
- (iii) Due to the presence of conformational defects of tetramers or decamers that lead to their prolonged ER retention and eventual slow degradation (see page 1489, left hand column). That is, because of the large size of the protein.
- (iiii) Due to saturation of secretion, because of the high level of synthesis. This was considered very unlikely on the basis of what is known about the process of secretion.

Furthermore, the Discussion of Frigerio states "*This raises the problem of which are the mechanisms and recognition events that lead to vacuolar delivery of a protein that is*

*expected to be secreted". The Discussion states "To be delivered to the vacuole, soluble proteins need sorting signals; when these signals are deleted, the mutated proteins are secreted, albeit with variable efficiencies (Bednarek et al., 1990, Crofts et al., 1990. Although one potential receptor for vacuolar sorting has been identified, the mechanisms for vacuolar delivery are not yet fully clarified, and certainly more than one mechanism exists (Vitale and Raikhel, 1999). However, we were unable to demonstrate tight binding of IgA/G to endomembranes." This clearly shows that Frigerio had doubts about whether a targeting mechanism existed.*

Thus it is clear that at the date of publication of Frigerio, the person skilled in the art did not know whether a targeting signal existed.

As a person skilled in the art of the field of this invention at the time of filing the present application, I would not have combined the teachings of Frigerio with those of Vitale, Koide or Matsuoka. This is because Vitale, Koide and Matsuoka discuss the expression of plant proteins in a plant. In contrast, Frigerio discusses the expression of a mammalian protein in a plant. The person skilled in the art would not expect a mammalian protein to possess a targeting signal which can be recognised for the delivery to plant vacuoles. Defective antibodies are disposed of in mammals by an entirely different route.

1. I expected IgA/G to cause endoplasmic reticulum stress, but this was experimentally ruled out.
2. I expected IgA/G to be subjected to quality control in the endoplasmic reticulum, with subsequent disposal in the vacuole. This however was also experimentally ruled out

Therefore, the identification of a targeting signal in a mammalian protein, as described in the present invention, is far from obvious.

In conclusion, I believe that the teachings of Frigerio, taken alone or in combination with Vitale, Koide and/or Matsuoka, would not have provided any motivation to generate the claimed invention, and would not render the present invention obvious. This is because it was surprising to discover that the underlying reason for heterologous mammalian protein being directed to the vacuole was due to the presence of a targeting signal. The degree of surprise is confirmed by the three years of research that was required to identify these facts.

I declare further that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of any patent issuing on this application.



Alessandro Vitale

18 October 2007

Date

**ALESSANDRO VITALE**  
**CURRICULUM VITAE AND PUBLICATIONS**

SURNAME:	Vitale
GIVEN NAME:	Alessandro
DATE OF BIRTH:	February 11, 1953
CITIZENSHIP:	Italian
MARITAL STATUS:	married, one son
AFFILIATION AND ADDRESS:	Istituto di Biologia e Biotecnologia Agraria (IBBA) CNR via Bassini 15 20133 Milano Italy tel: +39 02 23699431 fax: +39 02 23699411 E-mail: vitale@ibba.cnr.it

***Education, career and employment***

2002-present	Research Director, Istituto di Biologia e Biotecnologia Agraria (CNR, Agricultural Sciences). Field of activity: plant molecular and cell biology, plant biotechnology
1996-2001	Senior Researcher, Istituto di Biologia e Biotecnologia Agraria (CNR, Agricultural Sciences). Field of activity: plant molecular and cell biology
1986-96	Researcher, Istituto Biosintesi Vegetali - former denomination of IBBA - (CNR, Agricultural Sciences). Field of activity: plant molecular and cell biology
1982-83	Visiting scientist (awarded a CNR fellowship) in the laboratory of Maarten J. Chrispeels, UCSD, La Jolla, CA, to study the biosynthesis, intracellular transport and processing of bean phytohemagglutinin. Field of activity: plant cell biology
1982-86	Researcher, Istituto Biosintesi Vegetali, CNR Field of activity: plant biochemistry and cell biology
1978-82	Awarded a fellowship from CNR, Agricultural Sciences, to work at the Istituto Biosintesi Vegetali, Milano on the biochemical characterization and cell biology of maize storage proteins. Field of activity: plant biochemistry, cell biology and genetics
1971-76	Degree (Laurea) in Biology, Università degli studi di Milano. Mark: 110/110. Field: Biochemistry

***Teaching***

Academic years 2004- 2006	Professor on annual contract. University of Milano, for the Master Degree (laurea magistrale) in Plant Biotechnology .
Academic years 1998-2004	Professor on annual contract. University of Milano, for the Degree (laurea) in Plant Biotechnology .

***Responsibilities***

2006-09	Contractor of the European Commission Marie Curie Research Training Network "Vacuolar Transport Equipment for Growth Regulation in Plants" EU Sixth Framework Programme.
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- 2006-present Member of the Editorial Board of *Plant & Cell Physiology*
- 2004-07 Contractor of the Integrated Project "Recombinant Pharmaceuticals from Plants for Human Health". EU Sixth Framework Programme.
- 2003-05 Member of the Scientific Committee of the Istituto di Genetica Agraria, CNR
- 2003-05 Member of the Direction of the Italian Society of Plant Physiology
- 2002-05 Research Unit Leader within the project "Post genomics of forage legumes", supported by the Fondo per gli Investimenti della Ricerca di Base (FIRB) of the Ministry of Research of Italy.
- 2003 Member of the Italian delegation for the renewal of the Science and Technology Cooperation Agreement between Italy and the United States of America.
- 2002-06 Associated Contractor of the Research Training Network EU : "Biochemical and genetic approaches to study bio-molecular interactions in plants" EU Fifth Framework Programme.
- 1999-2004 Co-Editor of *The Plant Cell*
- 1997-2003 Editorial Consultant of *Plant Biosystems*
- 1997-2000 Member of the Scientific Committee of the Istituto biosintesi vegetali, CNR, Milano.
- 1997-2000 Member of the Scientific Committee of the Istituto di nematologia agraria applicata ai vegetali, CNR, Bari.
- 1995-97 Associated Contractor of the Project "Analysis of functional components and mechanisms controlling the plant endomembrane system", supported by the EU "Human Capital and Mobility" programme.
- 1994-present Editor of *Planta*
- 1994-present Member of the Advisory Board of *Journal of Experimental Botany*
- 1990-93 Research unit leader within the Progetto Finalizzato "Biotecnologie e Biostrumentazione" of the CNR. Project title: "Molecular biology of protein intracellular traffic and production of new proteins with determined locations".
- 1990-91 Research unit leader within the Progetto Speciale di Comitato del CNR "PS0408 – mechanisms responsible for the intracellular localization of proteins". Project title: "Assembly and intracellular traffic of phaseolin, the major storage protein of common bean"
- 1987-present Research Project leader at the Istituto di Biologia e Biotecnologia Agraria, CNR (formerly Istituto Biosintesi Vegetali). Research topics: seed storage proteins, molecular interactions and protein traffic within the plant secretory pathway, use of plants as bioreactors for the production of recombinant pharmaceuticals.

#### ***Honours, memberships***

- 2005 – present  
Member of the Italian Society for Agricultural Genetics (SIGA - Società Italiana di genetica Agraria)
- 1999 – present. Member of the American Society of Plant Biologists
- 1998 - Awarded the "Assunta Baccarini-Melandri" Prize of the Italian Society of Plant Physiology

1998 - present. Member of the Italian Society of Plant Physiologists (SIFV - Società Italiana di Fisiologia Vegetale)

***Plenary lecture speaker at International meetings (last three years)***

First Academia Belgica-Francqui Conference "Plants for the Future". Roma May 18, 2006. Title of presentation: Protein synthesis and accumulation in the plant secretory pathway.

Annual Meeting of the Korean Society for Molecular and Cellular Biology, October 17-18, 2005, Seoul, Korea. Title of presentation: Protein accumulation in the plant endoplasmic reticulum: relationships with protein quality control.

The Rank Prize Funds, Mini-Symposium on Improving the Plant Secretory System for Nutrition and Health. Grasmere, UK, 23rd to 26th May, 2005. Title of presentation: Functional and biochemical aspects.

**LIST OF PUBLICATIONS**

***Solicited papers***

Vitale, A. and Chrispeels, M.J. (1992) Sorting of proteins to the vacuoles of plant cells. **BioEssays** 14, 151-160. COVER STORY.

Vitale, A., Ceriotti, A. and Denecke, J. (1993) The role of the endoplasmic reticulum in protein synthesis, modification and intracellular transport. **J. Exp. Bot.** 44, 1417-1444.

Ceriotti, A., Pedrazzini, E., de Silvestris, M. and Vitale, A. (1995) Import into the Endoplasmic reticulum. Volume on "Plant Cell Biology" **Methods Cell Biol.** 50, part B, 295-308.

Denecke, J. and Vitale A. (1995) The use of protoplasts to study protein synthesis and transport by the plant endomembrane system. Volume on "Plant Cell Biology". **Methods Cell Biol.** 50, part B, 335-348.

Pedrazzini, E. and Vitale, A. (1996) The binding protein (BiP) and the synthesis of secretory proteins. **Plant Physiol. Biochem.** 34, 207-216.

Frigerio, L., Pedrazzini, E., Giovino, G., Bollini, R., Ceriotti, A. and Vitale, A. (1996) The synthesis of phaseolin: a model for the study of the plant secretory pathway. **Giorn. Bot. Ital.** 130, 891-900.

Vitale, A., and Denecke, J. (1999) The endoplasmic reticulum, gateway of the secretory pathway. **Plant Cell** 11, 615-628.

Vitale, A. and Raikhel, N.V. (1999) What do proteins need to reach different vacuoles?. **Trends Plant Sci.** 4, 149-155.

Vitale A. and Galili, G. (2001) The endomembrane system and the problem of protein sorting. **Plant Physiol.** 125, 115-118.

Vitale, A. (2001) Uncovering secretory secrets: inhibition of endoplasmic reticulum (ER) glucosidases suggests a critical role for ER quality control in plant growth and development. **Plant Cell** 13, 1260-1262.

Vitale, A. Physical methods. (2002) **Plant Mol. Biol.** 50, 825-836.

Vitale, A. and Ceriotti, A. (2004) Protein quality control mechanisms and protein storage in the endoplasmic reticulum. A conflict of interests? **Plant Physiol.** 136, 3420-3426.

Vitale, A. and Hinz, G. (2005) Sorting of proteins to storage vacuoles: how many mechanisms? *Trends Plant Sci.* 10, 316-323.

Vitale A. and Pedrazzini E. (2005) Recombinant pharmaceuticals from plants: the plant endomembrane system as bioreactor. *Mol. Interv.* 5, 216-225.

### **Research papers**

Longo, G.P., Longo, C.P., Rossi, G., Vitale, A. and Pedretti, M. (1978) Variations in carbohydrate and lipid content and in osmotic potential of watermelon cotyledons treated with benzyladenine. *Plant Sci. Lett.* 12, 199-207.

Vitale, A., Soave, C. and Galante, E. (1980) Peptide mapping of IEF zein components from maize. *Plant Sci. Lett.* 18, 57-64.

Bollini, R. and Vitale, A. (1981) Genetic variability in charge microheterogeneity and polypeptide composition of phaseolin, the major storage protein of *Phaseolus vulgaris*; and peptide maps of its major subunits. *Physiol. Plant.* 52, 96-100.

Vitale, A., Smaniotto, E., Longhi, R. and Galante, E. (1982) Reduced soluble proteins associated with maize endosperm protein bodies. *J. Exp. Bot.* 33, 439-448.

Bollini, R., Vitale, A. and Chrispeels, M.J. (1983) *In vivo* and *in vitro* processing of seed reserve protein in the endoplasmic reticulum: evidence for two glycosylation steps. *J. Cell Biol.* 96, 999-1007.

Galante, E., Vitale, A., Manzocchi, L., Soave, C. and Salamini, F. (1983) Genetic control of a membrane component and zein deposition in maize endosperm. *Mol. Gen. Genet.* 192, 316-321.

Chrispeels, M.J., Vitale, A. and Staswick, P. (1984) Gene expression and synthesis of phytohemagglutinin in the embryonic axes of developing *Phaseolus vulgaris* seeds. *Plant Physiol.* 76, 791-796.

Vitale, A., Ceriotti, A., Bollini, R. and Chrispeels, M.J. (1984a) Biosynthesis and processing of phytohemagglutinin in developing bean cotyledons. *Eur. J. Biochem.* 141, 97-104.

Vitale, A. and Chrispeels, M.J. (1984) Transient N-acetylglucosamine in the biosynthesis of phytohemagglutinin: attachment in the Golgi apparatus and removal in protein bodies. *J. Cell Biol.* 99, 133-140.

Vitale, A., Warner, T.G. and Chrispeels, M.J. (1984b) *Phaseolus vulgaris* phytohemagglutinin contains high-mannose and modified oligosaccharide chains. *Planta* 160, 256-263.

Chrispeels, M.J. and Vitale, A. (1985) Abnormal processing of the modified oligosaccharide sidechains of phytohemagglutinin in the presence of swainsonine and deoxynojirimycin. *Plant Physiol.* 78, 704-709.

Viotti, A., Cairo, G., Vitale, A. and Sala, E., (1985) Each zein gene class can produce polypeptides of different sizes. *EMBO J.* 4, 1103-1110.

Bollini, R., Ceriotti, A., Daminati, M.G. and Vitale, A. (1985) Glycosylation is not needed for the intracellular transport of phytohemagglutinin in developing *Phaseolus vulgaris* cotyledons and for the maintenance of its biological activities. *Physiol. Plant.* 65, 15-22.

Vitale, A., Ceriotti, A., Bollini, R. (1985) Molecular analysis of a phytohemagglutinin-defective cultivar of *Phaseolus vulgaris* L. *Planta* 166, 201-207.

Vitale, A., Sturm, A. and Bollini, R. (1986) Regulation of processing of a plant glycoprotein in the Golgi complex: a comparative study using *Xenopus* oocytes. *Planta* 169, 108-116.

Faye, L., Sturm, A., Bollini, R., Vitale, A. and Chrispeels, M.J. (1986) The position of the oligosaccharide side-chains of phytohemagglutinin and their accessibility to glycosylases determines their subsequent processing in the Golgi. *Eur. J. Biochem.* 158, 655-661.

Fabbrini, M.S., Zoppè, M., Bollini, R. and Vitale, A. (1988) 1-deoxymannojirimycin inhibits Golgi-mediated processing of glycoprotein in *Xenopus* oocytes. *FEBS Lett.* 234, 489-492.

Vitale, A., Zoppè, M. and Bollini, R. (1989a) Mannose analog 1-deoxymannojirimycin inhibits the Golgi-mediated processing of bean storage glycoproteins. *Plant Physiol.* 89, 1079-1084.

Vitale, A., Zoppè, M., Fabbrini, M.S., Genga, A., Rivas, L., Bollini, R. (1989b) Synthesis of lectin-like protein in developing cotyledons of normal and phytohemagglutinin-deficient *Phaseolus vulgaris*. *Plant Physiol.* 90, 1015-1021.

Cerioti, A., Vitale, A. and Bollini, R. (1989) Lectin-like proteins accumulate as fragmentation products in bean seed protein bodies. *FEBS Lett.* 250, 157-160.

Benatti, L., Nitti, G., Solinas, M., Valsasina, B., Vitale, A., Cerioti, A. and Soria, M.R. (1991) A saporin-6 cDNA containing a precursor sequence coding for a carboxyl-terminal extension. *FEBS Lett.* 291, 285-288.

Cerioti, A., Pedrazzini, E., Fabbrini, M.S., Zoppè, M., Bollini, R. and Vitale, A. (1991) Expression of wild-type and mutated vacuolar storage protein phaseolin in *Xenopus* oocytes reveals relationships between assembly and intracellular transport. *Eur. J. Biochem.* 202, 959-968.

Fabbrini, M.S., Valsasina, B., Nitti, G., Benatti, L., Vitale, A. (1991a) The signal peptide of human preproendothelin-1. *FEBS Lett.* 286, 91-94.

Fabbrini, M.S., Vitale, A., Patrono, C., Zamai, M., Vaghi, F., Caiola, V., Monaco, L. and Benatti, L. (1991b) Heterologous *in vivo* processing of human preproendothelin-1 into bioactive peptides. *Proc. Natl. Acad. Sci. USA* 88, 8939-8943.

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D'Amico, L., Valsasina, B., Daminati, M.G., Fabbrini, M.S., Nitti, G., Bollini, R., Cerioti, A. and Vitale, A. (1992) Bean homologs of the mammalian glucose regulated proteins: induction by tunicamycin and interaction with newly synthesized storage proteins in the endoplasmic reticulum. *Plant J.* 2, 443-455.

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Fabbrini, M.S., Vitale, A., Pedrazzini, E., Nitti, G., Zamai, M., Tamburin, M., Caiola, V.R., Patrono, C. and Benatti, L. (1993) *In vivo* expression of mutant preproendothelins: Hierarchy of processing events but no strict requirement of Trp-Val at the processing site. *Proc. Natl. Acad. Sci. USA* 90, 3923-3927.

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Baldan, B., Guzzo, F., Filippini, F., Gasparian, M., LoSchiavo, F., Vitale, A., de Vries, S., Mariani, P. and Terzi, M. (1997) The secretory nature of the lesion of carrot cell variant ts11, rescuable by endochitinase. *Planta*, 203, 381-389.

Giovinazzo, G., Greco, V., Vitale, A. and Bollini, R. (1997) Bean (*Phaseolus vulgaris* L.) protoplasts as a model system to study the expression and stability of recombinant seed proteins. *Plant Cell Rep.* 16, 705-709.

Pedrazzini E., Giovinazzo, G., Bielli, A., de Virgilio, M., Frigerio, L., Pesca, M., Faoro, F., Bollini, R., Ceriotti, A. and Vitale, A. (1997) Protein quality control along the route to the plant vacuole. *Plant Cell* 9, 1869-1880.

Crofts, A., Leborgne-Castel, N., Pesca, M., Vitale, A., and Denecke, J. (1998) BiP and calreticulin form an abundant complex that is independent of endoplasmic reticulum stress. *Plant Cell* 10, 813-823.

Frigerio, L., Vitale, A., Lord, J.M., Ceriotti, A. and Roberts, L.M. (1998) Free ricin A chain, proricin, and native toxin have different cellular fates when expressed in tobacco protoplasts. *J. Biol. Chem.* 273, 14194-14199.

Frigerio, L., de Virgilio, M., Prada, A., Faoro, F., and Vitale, A. (1998) Sorting of phaseolin to the vacuole is saturable and requires a short C-terminal peptide. *Plant Cell* 10, 1031-1042.

Frigerio, L., Vine, N.D., Pedrazzini, E., Hein, M.B., Wang, F., Ma, J. K-C., and Vitale, A. (2000) Assembly, secretion and vacuolar delivery of a hybrid immunoglobulin in plants. *Plant Physiol.* 123, 1483-1493.

Frigerio, L., Foresti, O., Hernández Felipe, D., Neuhaus, J-M., and Vitale, A. (2001) The C-terminal tetrapeptide of phaseolin is sufficient to target green fluorescent protein to the vacuole. *J. Plant Physiol.* 158, 499-503.

Frigerio, L., Pastres, A., Prada, A., and Vitale, A. (2001) Influence of KDEL on the fate of trimeric or assembly-defective phaseolin: selective use of an alternative route to vacuoles. *Plant Cell* 13, 1109-1126.

Holkeri, H. and Vitale, A. (2001) Vacuolar sorting determinants within a plant storage protein trimer act cumulatively. *Traffic* 2, 737-741.

Nuttall, J., Vitale, A., and Frigerio, L. (2003) C-terminal extension of phaseolin with a short methionine-rich sequence can inhibit trimerisation and result in high instability. *Plant Mol. Biol.* 51, 885-894.

Hadlington, J.L., Santoro, A., Nuttall, J., Denecke, J., Ma, J.K-C., Vitale, A., and Frigerio, L. (2003) The C-terminal extension of a hybrid immunoglobulin A/G heavy chain is responsible for its Golgi-mediated sorting to the vacuole. *Mol. Biol. Cell* 14, 2592-2602.

Foresti, O., Frigerio, L., Holkeri, H., de Virgilio, M., Vavassori, S., and Vitale, A. (2003) A phaseolin domain directly involved in trimer assembly is a BiP binding determinant. *Plant Cell* 15, 2464-2475.

Park, M., Kim, S.J., Vitale, A., and Hwang I. (2004) Identification of the protein storage vacuole and protein targeting to the vacuole in leaf cells of three plant species. *Plant Physiol.* 134, 625-639.

Cervelli, M., Di Caro, O., Di Penta, A., Angelini, R., Federico, R., Vitale, A. and Mariottini, P. (2004) A novel C-terminal sequence from barley polyamine oxidase is a vacuolar sorting signal. *Plant J.* 40, 410-418.

Mainieri, D., Rossi, M., Archinti, M., Bellucci, M., De Marchis, F., Vavassori, S., Pompa, A., Arcioni, S. and Vitale A. (2004) Zeolin. A new recombinant storage protein constructed using maize gamma-zein and bean phaseolin. *Plant Physiol.* 136, 3447-3456.

Castelli, S. and Vitale, A. (2005) The phaseolin vacuolar sorting signal promotes transient, strong membrane association and aggregation of the bean storage protein in transgenic tobacco. *J. Exp. Bot.* 56, 1379-1387.

Pompa, A. and Vitale, A. (2006) Retention of a bean phaseolin/maize  $\gamma$ -zein fusion in the endoplasmic reticulum depends on disulfide bond formation. *Plant Cell* 18, 2608-2621.

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